

# CLAIMS

1            1. A ligand profile which is characteristic for a ✓  
2 given cell, the ligand profile comprising a representation  
3 of at least ten different polypeptide ligands, all of which  
4 bind to a single type of multi-ligand binding receptor,  
5 wherein the representation characterizes each individual  
6 ligand based upon at least three physical or chemical  
7 attributes; provided that, if the multi-ligand binding  
8 receptor is an MHC class I or class II receptor, at least  
9 500 polypeptide ligands are represented in the ligand  
10 profile; and further provided that the ligand profile is a  
11 reproducible characteristic of the cell.

1            2. A ligand profile which is characteristic for a ✓  
2 given cell, the ligand profile comprising a representation  
3 of at least ten different polypeptide ligands, all of which  
4 bind to a single type of multi-ligand binding receptor,  
5 wherein the representation characterizes each individual  
6 ligand based upon at least two physical or chemical  
7 attributes, one of said attributes being mass or mass-to-  
8 charge ratio; provided that, if the multi-ligand binding  
9 receptor is an MHC class I or class II receptor, at least  
10 500 polypeptide ligands are represented in the ligand  
11 profile; and further provided that the ligand profile is a  
12 reproducible characteristic of the cell.

1            3. A ligand profile which is characteristic for a ✓  
2 given cell, the ligand profile comprising a representation  
3 of at least ten different polypeptide ligands, all of which  
4 bind to a single type of multi-ligand binding receptor,  
5 wherein the representation characterizes each individual  
6 ligand based upon at least one physical or chemical  
7 attribute, the at least one physical or chemical attribute



1 <sup>SWB 3)</sup> 7. The ligand profile of claim 1, wherein the  
2 multi-ligand binding receptor is not an MHC class I or MHC  
3 class II receptor

1 <sup>SWB 1)</sup> 8. The ligand profile of claim 1, wherein the  
2 multi-ligand binding receptor is a chaperone, a chaperonin,  
3 a calnexin, a calreticulin, a mannosidase, a N-glycanase, a  
4 BIP, a grp94, a grp96, hsp60, hsp65, hsp70, hsp90, hsp25, an  
5 E2 ubiquitin carrier protein, an E3 ubiquitin ligase, an  
6 unfoldase, hsp100, a proteasome, a trafficking protein, or a  
7 retention protein.

1 9. The ligand profile of claim 1, combined with a  
2 second ligand profile, the second ligand profile (a) also  
3 being a reproducible characteristic of the given cell, and  
4 <sup>SWB 3)</sup> (b) comprising a representation of at least ten additional  
5 polypeptide ligands, all of which bind to a second type of  
6 multi-ligand binding receptor different from the first type  
7 of receptor.

1 <sup>SWB 2)</sup> 10. A method of generating a reproducible ligand  
2 profile for a given cell type, which cell type comprises a  
3 selected type of multi-ligand binding receptor, the method  
4 comprising:  
5 (a) providing a first sample of the given cell  
6 type, wherein the first sample comprises a first plurality  
7 of polypeptide ligands bound to the selected type of multi-  
8 ligand binding receptor;  
9 (b) isolating the selected type of multi-ligand  
10 binding receptor from the first sample;  
11 (c) separating the first plurality of ligands from  
12 the selected type of multi-ligand binding receptor;  
13 (d) fractionating the first plurality of ligands;

14 (e) generating a first profile distinguishing among  
15 the first plurality of ligands on the basis of at least one  
16 chemical or physical attribute;

17 (f) providing a second sample of the given cell  
18 type, the second sample being essentially identical to the  
19 first sample, wherein the second sample comprises a second  
20 plurality of polypeptide ligands bound to the selected type  
21 of multi-ligand binding receptor;

22 (g) isolating the selected type of multi-ligand  
23 binding receptor from the second sample;

24 (h) separating the second plurality of ligands from  
25 the selected type of multi-ligand binding receptor;

26 (i) fractionating the second plurality of ligands;

27 (j) generating a second profile distinguishing  
28 among the second plurality of ligands on the basis of the at  
29 least one chemical or physical attribute; and

30 (k) confirming that the first profile and the  
31 second profile are essentially identical, and together  
32 represent a reproducible ligand profile for the given cell  
33 type.

1 11. The method of claim 10, wherein a second  
2 chemical or physical attribute of each ligand is determined  
3 subsequent to the fractionation steps, and is represented in  
4 the profiles.

1 12. The method of claim 11, wherein a third  
2 chemical or physical attribute of each ligand is determined  
3 subsequent to the fractionation steps, and is represented in  
4 the profiles.

1 13. The method of claim 10, wherein the isolating  
2 and separating steps are accomplished using appropriate  
3 columns arranged in an in-line system.

1 14. A method of generating a ligand profile for a  
2 given type of cell, comprising:

3 (a) providing a sample of lysate of the given type  
4 of cell, wherein the sample comprises a first plurality of  
5 polypeptide ligands bound to a first type of multi-ligand  
6 binding receptor and a second plurality of polypeptide  
7 ligands bound to a second type of multi-ligand binding  
8 receptor;

9 (b) isolating the first and second types of multi-  
10 ligand binding receptors from the sample;

11 (c) separating the first plurality of ligands from  
12 the first type of multi-ligand binding receptor and the  
13 second plurality of ligands from the second type of multi-  
14 ligand binding receptor;

15 (d) fractionating the first plurality of ligands  
16 and the second plurality of ligands; and

17 (e) generating a first profile distinguishing among  
18 the first plurality of ligands on the basis of at least one  
19 chemical or physical attribute and a second profile  
20 distinguishing among the second plurality of ligands on the  
21 basis of the same at least one chemical or physical  
22 attribute.

1 15. A method of generating a subtraction profile of  
2 polypeptide ligands, comprising:

3 (a) producing a first ligand profile by a method  
4 comprising:

5 (i) providing a first sample comprising a  
6 first cell of interest, wherein the first cell of interest



3 (a) producing a first ligand profile by a method  
 4 comprising:  
 5 (i) providing a first cell sample comprising  
 6 a given type of multi-ligand binding receptor bound to a  
 7 first set of polypeptide ligands;  
 8 (ii) isolating the given type of multi-ligand  
 9 binding receptor and the first set of ligands from the first  
 10 cell sample;  
 11 (iii) separating the first set of ligands from  
 12 the given type of multi-ligand binding receptor;  
 13 (iv) generating a first ligand profile  
 14 distinguishing among the first set of ligands on the basis  
 15 of at least one chemical or physical attribute;  
 16 (b) providing a reference ligand profile  
 17 representing a second set of polypeptide ligands extracted  
 18 from the given type of multi-ligand binding receptor of a  
 19 reference cell sample, wherein the reference ligand profile  
 20 distinguishes among the second set of polypeptide ligands on  
 21 the basis of the at least one chemical or physical  
 22 attribute; and  
 23 (c) comparing the first ligand profile to the  
 24 reference ligand profile, in order to identify differences  
 25 or similarities between the first cell sample and the  
 26 reference cell sample.

1 18. The method of claim 17, wherein the reference  
 2 cell sample consists essentially of healthy cells of an  
 3 animal and the first cell sample comprises cells suspected  
 4 of being diseased.

1 19. The method of claim 17, wherein the first cell  
 2 sample comprises cells cultured in the presence of a test  
 3 compound, and the reference cell sample does not.

1           20. The method of claim 17, wherein the reference  
2 cell sample comprises cells cultured in the presence of a  
3 test compound, and the first cell sample does not.

1           21. A set of ligand profiles, comprising  
2       sub 5) (a) a first ligand profile comprising a first  
3 representation of a first plurality of polypeptide ligands,  
4 all of which bind to at least one multi-ligand binding  
5 receptor of a first cell, wherein the first representation  
6 distinguishes among the members of the first plurality of  
7 ligands based upon at least one physical or chemical  
8 attribute; and

9           (b) a second ligand profile comprising a second  
10 representation of a second plurality of polypeptide ligands,  
11 all of which bind to the at least one type of multi-ligand  
12 binding receptor of a second cell, wherein the second  
13 representation distinguishes among the second plurality of  
14 ligands based upon the at least one physical or chemical  
15 attribute;  
16 provided that (i) the first cell differs from the second  
17 cell in a parameter selected from the group consisting of  
18 genetic background, culture conditions, genetic background  
19 plus culture conditions, *in vivo* exposure to a test  
20 compound, and genetic background plus *in vivo* exposure to a  
21 test compound; and (ii) any significant difference between  
22 the first and the second ligand profiles is attributable to  
23 that parameter.

1           22. A method of detecting a difference between the  
2 set of proteins expressed in a first cell and the set of  
3 proteins expressed in a second cell, comprising  
4           (a) providing a first ligand profile made by a  
5 method comprising

(i) providing a first cell comprising at least one type of multi-ligand binding receptor, bound to a first set of polypeptide ligands,

(ii) isolating from the first cell the at least one type of multi-ligand binding receptor bound to the first set of ligands,

(iii) separating the first set of ligands from the at least one type of multi-ligand binding receptor, and

(iv) generating a first ligand profile distinguishing among the members of the first set of ligands on the basis of at least one chemical or physical attribute;

(b) providing a second ligand profile made by a method comprising

(i) providing a second cell comprising the at least one type of multi-ligand binding receptor, bound to a second set of polypeptide ligands,

(ii) isolating from the second cell the at least one type of multi-ligand binding receptor, bound to the second set of ligands,

(iii) separating the second set of ligands from the at least one type of multi-ligand binding receptor, and

(iv) generating a second ligand profile distinguishing among the members of the second set of ligands on the basis of the at least one chemical or physical attribute;

(c) comparing the first ligand profile to the second ligand profile, in order to identify any difference between the first and second profiles, wherein such a difference is an indication of a difference between the set of proteins expressed in the first cell and the set of proteins expressed in the second cell.





5 receptor types, the database defining associations among  
 6 instances of the three categories of data, the query  
 7 comprising one or more instances of one of the three  
 8 categories of data, and  
 9 applying the query to the database to find instances  
 10 of another one of the three categories of data.

1 36. The method of claim 35 in which the found  
 2 instances comprise two ligand profiles.

1 37. The method of claim 36 further comprising  
 2 comparing the two ligand profiles to determine a  
 3 difference between them.

1 38. The method of claim 36 in which the query  
 2 comprises instances of a selected cell source comprising a  
 3 selected cell condition.

1 39. A machine-based method comprising  
 2 performing an experiment on cells,  
 3 identifying a ligand profile associated with said  
 4 cells, and  
 5 based on the ligand profile, querying a database  
 6 that contains at least two categories of data, including  
 7 ligand profiles and cell sources, to derive a cell source or  
 8 a ligand profile and an associated cell source.

1 40. The method of claim 39 in which  
 2 the feature of the experiment comprises treatment of  
 3 the cells using a candidate drug regimen, and  
 4 a cell source identified as a result of the query  
 5 represents a different treatment of cells.

41. A machine-assisted method of investigation  
comprising  
identifying a cell source, a receptor type, or a  
ligand profile of interest, and  
based on the identified cell source, receptor type,  
or ligand profile, querying a database that contains three  
associated categories of data respectively representing (a)  
ligand profiles, (b) cell sources, and (c) receptor types,  
to derive information about cell sources, receptor types, or  
ligand profiles that relates to the cell source, receptor  
type, or ligand profile of interest.

42. A machine-assisted method comprising  
providing cells of a cell source,  
generating a ligand profile from the cells, and  
based on the ligand profile and the cell source,  
querying a database that contains three associated  
categories of data respectively representing (a) ligand  
profiles, (b) cell sources, and (c) receptor types, to  
derive information about cell sources, receptor types, or  
ligand profiles that relates to the provided cell source and  
the generated ligand profile.

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